Perchlorate

Results of the Perchlorate Study Protocol Review Meeting

Perchlorate Study Protocol Peer Review May 20, 1997 Summary Meeting Notes

REVIEW PANEL

- Dr. Joe Brown, California EPA, Office of Environmental Health Hazard Assessment
- Dr. Dan Caldwell, Toxicologist, Belle Meade, NJ
- Dr. Dorothy Canter, U.S. EPA, Office of Solid Waste and Emergency Response
- Dr. Charles Capen, Ohio State University, Department of Veterinary Biomedicine
- Dr. John Christopher, California EPA, Department of Toxic Substances Control
- Dr. Marvin Friedman, Cytec Industries, Inc.
- Mr. Greg Harvey, U.S. Air Force, Wright-Patterson Air Force Base
- Ms. Annie Jarabek, U.S. EPA, National Center for Environmental Assessment
- Dr. David Morry, California EPA, Office of Environmental Health Hazard Assessment
- Dr. Marilyn Underwood, California Department of Health Services

On March 7, 1997, an International Toxicity Estimates for Risk (ITER) Peer Review Panel concluded that the database for perchlorate was insufficient for development of a reference dose (RfD) and that additional studies should be conducted. As a result of this recommendation, the Perchlorate Study Group and the U.S. Air Force obtained funding to initiate studies on perchlorate. The purpose of the May 20, 1997 meeting was to develop a prioritized list of toxicological studies that could be performed, and to provide guidance on the development of protocols that would be used for those studies. This would ensure that the data generated are useful in developing a reference dose. These notes provide a summary of the May 20 panel conclusions. The protocols will be available, once the contract laboratories are selected, on the Toxicology Excellence for Risk Assessment (TERA) home page (www.tera.org/news).

The panel concluded that the following types of studies/toxicities were the most important to study:

1. Neurobehavioral Developmental

Areas of Scientific Uncertainty: Avg. Human to Sensitive Human and Data Base

Critical Effect: Neurobehavioral deficits due to toxicity to developing thyroid?

2. 90-day, all other organs

Areas of Scientific Uncertainty: Data Base

Critical Effect: Thyroid as critical effect?

3. Receptor Kinetics (*in vitro* studies)

Areas of Scientific Uncertainty: Avg. Human to Sensitive Human, Animal to Human

Critical Effect: Comparative human and rat discharge data?

4. Segment II Developmental

Areas of Scientific Uncertainty: Data Base

Critical Effect: Gross and skeletal fetal abnormalities, fetal survival

5. ADME - Absorption, Distribution, Metabolism and Elimination

Areas of Scientific Uncertainty: Avg. Human to Sensitive Human, Animal to Human and Data Base

Critical Effect: Comparative studies must be done carefully

6. Mutagenicity/Genotoxicity

Areas of Scientific Uncertainty: Short Term to Long Term Studies, Data Base

7. Reproductive

Areas of Scientific Uncertainty: Data Base

8. Immunotoxicity

Areas of Scientific Uncertainty: Data Base

Of these eight areas, it is essential to complete the 90-day study and the neurobehavioral developmental study in order to determine that the critical effect(s) of perchlorate has been identified. The remaining studies are useful for addressing a number of other uncertainties in the database. In addition, an in-depth review of the current literature on the toxicity of perchlorate

compounds and on the comparative sensitivity of humans and rats to thyroid toxicants was recommended by the panel. This is currently in progress at Wright-Patterson Air Force Base. With this information, prepared (and once the present set of studies have been completed a complete summary of toxicological data can be). This will also enable the development of a proposed RfD on a more informed basis.

STUDY DESIGN ISSUES

Studies should be conducted using ammonium perchlorate (the contaminant of concern at hazardous waste sites) in drinking water; provided that the ammonium ion remains stable and does not degrade to nitrate. One reviewer suggested; however, dosing in the diet for the neurobehavioral developmental study, since an increase in drinking water consumption by lactating rats would cause an increase in the actual dose received. In addition, the study protocols should specify randomization of cages and twice daily observations for clinical signs.

1. Neurobehavioral Developmental Study

Doses: 10.0, 3.0, 1.0, 0.1, 0 mg/kg-day in drinking water in rats. Start dosing at confirmed day one of pregnancy and dose through postnatal day (PND) 10. (Need to state in final protocol that dosing earlier than normal in order to ensure that dams are hypothyroid). Sacrifice five dams per group for thyroid parameters (TSH, T3, T4, thyroid weight) at PND10. One reviewer also suggested that reverse T3 as an indicator of T4 metabolism and prolactin because it is regulated with TSH should also be measured as thyroid parameters. The U.S. EPA Office of Pollution Prevention and Toxic Substances (OPPTS) Health Effects Guidelines call for a learning test (either the same or two different tests) at two time points (at weaning and as adults). A neurotoxicology expert has been consulted to determine if the same test should be done at both time points and if two learning tests will provide better understanding of potential perchlorate effects on learning in human infants.

Use glutaraldehyde fixation of tissues when sacrificing animals. One reviewer suggested requesting three dimensional microscopy for the neurological tissue to distinguish possible effects on nerve cell number from effects on nerve cell size and volume. Other reviewers suggested that this would only be necessary if traditional microscopy revealed effects on nerve tissue. The EPA Health Effects Guidelines will be followed to determine the number of dams in each treatment group, the number of pups from dams from each treatment group to be tested for neurobehavioral indices, and the manner in which pups will be selected from litters.

2. 90-day study

Doses: 10.0, 1.0, 0.2, 0.05, 0.01, 0 mg/kg-day in drinking water with twenty rats/sex/group. Ten rats/sex/group will be sacrificed at 14 days for measurement of thyroid parameters, based upon the hypothesis that most toxicity to the thyroid is seen during this time period Ten rats/sex/group to be sacrificed at 90 days. An additional 10 animals/sex/group treated for 90 days at dose levels of 10, 1, 0.05, and 0 mg/kg/day will be maintained without further treatment for 30 more days to ascertain whether there is any recovery from toxic effects seen at 90 days. One reviewer

suggested that since the key question to be answered by the recovery group was the reversibility of the thyroid effects after exposure stopped, that only control and high dose were needed to answer that question.

Thyroid parameters (TSH, T3, T4, thyroid weight) to be measured at 14, 90 and 120 days. One reviewer also suggested including reverse T3 as an indicator of T4 metabolism and prolactin because it is regulated with TSH. Clinical chemistry and hematology to be performed at 45 and 90 days. Organ weights (including heart) and histopathology (including heart) evaluated at 90 days and at the end of the recovery period. Control and high dose groups will be evaluated; if pathological effects attributed to ammonium perchlorate are observed, remaining dose groups will be evaluated. Immunotoxicity will be assessed at day 90 by measuring response in the sheep red blood cell (SRBC) assay. Dominant lethal assay will be conducted at 90 days.

3. Receptor kinetics (*in vitro* studies).

Perchlorate Discharge Test has been conducted in both rats and humans, which could provide sufficient information about relative sensitivities in both species. Prior to initiating any new studies, the existing literature should be reviewed to ascertain the need for additional studies and to design better studies, if such are needed.

4. Developmental

Full determination of skeletal anomalies is critical for completing the database on this endpoint. If this is not done as part of the developmental neurological study, then a Segment II (teratogenicity) would be necessary. However, one reviewer suggested that if the results of the developmental neurological study are negative and there are no obvious birth defects observed in that study, then a Segment II study is not necessary. The rat is the preferred species

5. ADME - Absorption, Distribution, Metabolism and Elimination

Tissue uptake, half-life in serum, metabolic products, and excretion of labeled perchlorate would be measured in rats. These studies would aid in extrapolation across species (and in selecting the most appropriate uncertainty factor to account for that extrapolation). No data exist for ammonium perchlorate and some insight may be gained by comparing the results of the perchlorate discharge test in both rats and humans (see above). However, it was noted that this test does not distinguish between pharmacokinetics and pharmacodynamics.

6. Mutagenicity/Genotoxicity

Dominant lethal at end of the 90-day study will address in vivo genotoxicity. A separate Ames test should be conducted in standard strains to assess potential for both base-pair and frameshift mutations, with and without metabolic activation. A genetic toxicity expert has been consulted to determine if other tests (sister chromatid exchange in Chinese hamster ovary [CHO], Micronucleus) are appropriate or redundant. A micronucleus test was suggested in addition to the dominant lethal.

7. Reproductive

Consider this study after the results of the 90-day and neurobehavioral developmental studies are available.

8. Immunotoxicity

Sheep RBC screen is part of the 90-day study. Consider other immunotoxicity assays after the results of the 90-day and neurobehavioral developmental studies are available.

Return to TERA News

Return to TERA Homepage
